

Electronic Supporting Information (ESI)

A new method for determination of total furanic aldehydes compounds in Brazilian cachaça samples using liquid-liquid extraction and UV detection

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Experimental

Chromatograms and some quantitative data from reference GC-FID method

Sample preparation: Brazilian cachaça samples were steam-distilled for a few minutes, and an aliquot of the distillate was taken for determine the actual alcohol content in each sample. It was performed by densimetry using a hydrostatic balance. Right after, 50:50 ethanol-water mixtures (v/v) were prepared using the remaining sample distillate, trying to match the characteristics of the sample matrix.

Furfural standard solutions preparation: the furfural stock standard solution (990 mg L⁻¹) was diluted more than 400 times in order to obtain seven furfural working standard solutions ranging 0.30 – 5.50 mg mL. N-pentanol was used as internal standard in both sample distillates and furfural standard solutions, with the same amount added to each sample/reference standard mixture to normalize the results to a common basis. More detailed GC-FID set up data is already described in main text.

Chromatograms/Calculations: each furfural standard solution was treated in the same way as the samples, including the addition of internal standard. Its chromatograms were obtained at the beginning of each group of samples analyzed, and the peak areas used to calculate response factors (RF) relative to N-pentanol:

$$RF(fur + HMF) = \left[\frac{area(ISTD)}{amt(ISTD)} \right] / \left[\frac{area(fur + HMF)}{amt(fur)} \right] \quad \text{Eq. 1}$$

where: area(ISTD) = area of the internal standard peak; amt (ISTD) = amount of the internal standard added to the reference mixture; area(fur+HMF) = sum of area peaks of furfural and Hydroxy methylfurfural (HMF); and amt(fur) = amount of furfural in the

reference mixture. Using RF values, we calculated the amounts of furfural in the samples as follows:

$$w(fur) = \frac{RF(fur + HMF) \cdot area(fur + HMF) \cdot amt(ISTD) \cdot DF}{area(ISTD) \cdot w_{sample}} \quad \text{Eq. 2}$$

$w(fur)$ = mass of furfural per g of sample; $area(fur+HMF)$ = Sum of areas of furfural and HMF in the chromatogram of the sample; $amt(ISTD)$ = amount of the internal standard added to the sample; $area(ISTD)$ = area of the internal standard in the chromatogram of the sample; DF = dilution factor and w_{sample} = mass of sample expressed in g.

Note that HMF and furfural are quantified as just one analyte according with reference method adopted in Brazil. For this reason, the area peaks of both furfural and HMF are summed. Chromatograms of two cachaça samples are shown in Figures ESI1 to ESI3.

[Insert Figure ESI 1 here]

[Insert Figure ESI 2 here]

[Insert Figure ESI 3 here]

Figure ESI1 shows a chromatogram of a sample distillate with absence of furfural and peaks of several secondary components. Figures ESI2 and ESI3 show chromatograms of a reference standard mixture spiked with furfural and of a sample spiked with furfural, respectively. Peak of internal standard (N-pentanol) is indicated as ISTD. GC-FID retention time for furfural was round 13.4 minutes and no peak of HMF was detected on these distilled samples. All peaks were completely separated or separated well enough for quantitative estimation. Figure ESI4 shows results from plotting of amount and area ratios, some curve parameters and RF values used in quantitative calculations.

[Insert Figure ESI 4 here]

These data were obtained from chromatographic peaks of a reference standard mixture spiked with furfural in seven levels, showed in Figure ESI5. The areas of each furfural peaks are given in arbitrary units. All information above were gently provided by ITEP (Instituto de Tecnologia de Pernambuco), Pernambuco, Brazil.

[Insert Figure ESI 5 here]

Residual chloroform recovery

The treatment of the residual chloroform was conducted according to a method described in the literature (EMBRAPA, 2014A). The method is based on the chloroform filtration with a bottom layer of neutral alumina (activated and kiln-dried at 110 °C for 24 hours) and a top layer of glass wool under reduced pressure. After filtration, the chloroform is then stirred overnight with some sodium hydroxide lentils (NaOH).

Afterwards, chloroform is filtered again to remove hydroxide lentils using a glass funnel with glass wool and then proceeds successive washings with Milli-Q grade water (1: 1 ratio v/v) successively until pH of the aqueous phase is between 5 and 6. The solvent is finally stored in amber vial containing anhydrous sodium sulfate as drying agent, and then distilled by fractional distillation. Spectra form chloroform before and after treatment are showed in Figure ESI6.

[Insert Figure ESI 6 here]

Solvent study

For a quantitative analysis, a solvent should be spectrally “transparent” in the spectral region under investigation; it can be evaluated by solvent UV-Vis absorbance cut-off (L_1). L_1 is the wavelength below which the solvent itself absorbs all of the light when a 1.0 cm cuvette filled with pure solvent, whose absorbance does not exceed 1.0 absorbance units (relative to water). So when choosing a solvent, the researcher needs to

observe that the wavelength cut-off should be as low as possible in the measured range. Figure ESI7 illustrates the cut-off wavelengths for pure chloroform and pure ethanol against water as the blank.

[Insert Figure ESI 7 here]

The cut-off wavelength for chloroform was found to be 244 nm (black line), very close to values reported in books and handbooks and it did not influence the measurements in the absorption band of furfural/HMF (red line). Low amounts of ethanol are present inside bulk extracts bulk due to joint co-extraction with furfural, so L_1 for ethanol was also evaluated (207 nm) (blue line). As can be seen, neither ethanol nor chloroform affected any spectrometric measurement above 250 nm.

Captions for Figures

Figure ESI1: Chromatogram of a distillate from a Brazilian cachaça sample tested negatively for TFA.

Figure ESI2: Chromatogram of a reference standard mixture spiked with furfural and n-pentanol as internal standard (ISTD).

Figure ESI3: Chromatogram of a distillate from a Brazilian cachaça sample tested positively for furfural and spiked with internal standard n-pentanol.

Figure ESI4: Screen capture taken from control program of the GC-FID instrument (gas chromatograph model GC 2014 Shimadzu). Here are listed the amount and peak

area ratios; RF values and curve parameters obtained from a set of seven spike levels on a reference standard mixture.

Figure ESI5: Chromatographic peaks of seven spike levels on a reference standard mixture. The numbers in yellow are the peak areas given in arbitrary units.

Figure ESI6: UV Spectra from residual chloroform before (green curve) and after (red curve) treatment procedure. Pure chloroform was used as blank solution.

Figure ESI7: Cut-off wavelengths for pure chloroform (black line) and ethanol (blue line) and a spectrum of a chloroform extract (red line) enriched with furfural (10.0 mg 100 mL⁻¹). For all solutions, absorption spectra were recorded in the range from 190.0 to 330.0 nm against the same blank (water).

Figure ESI1

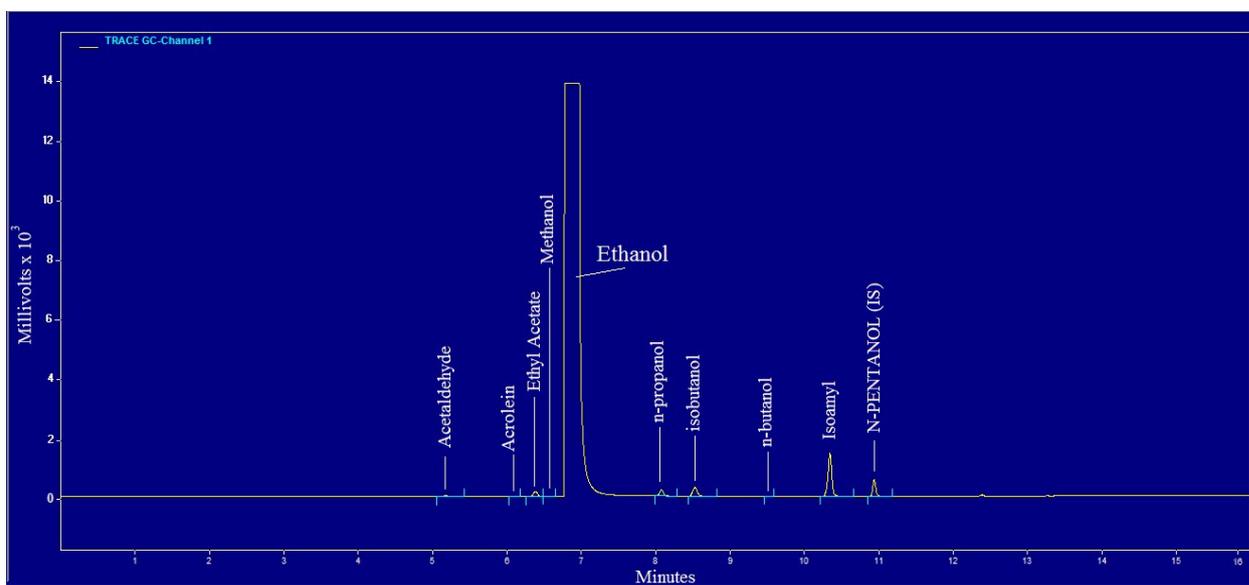


Figure ESI2

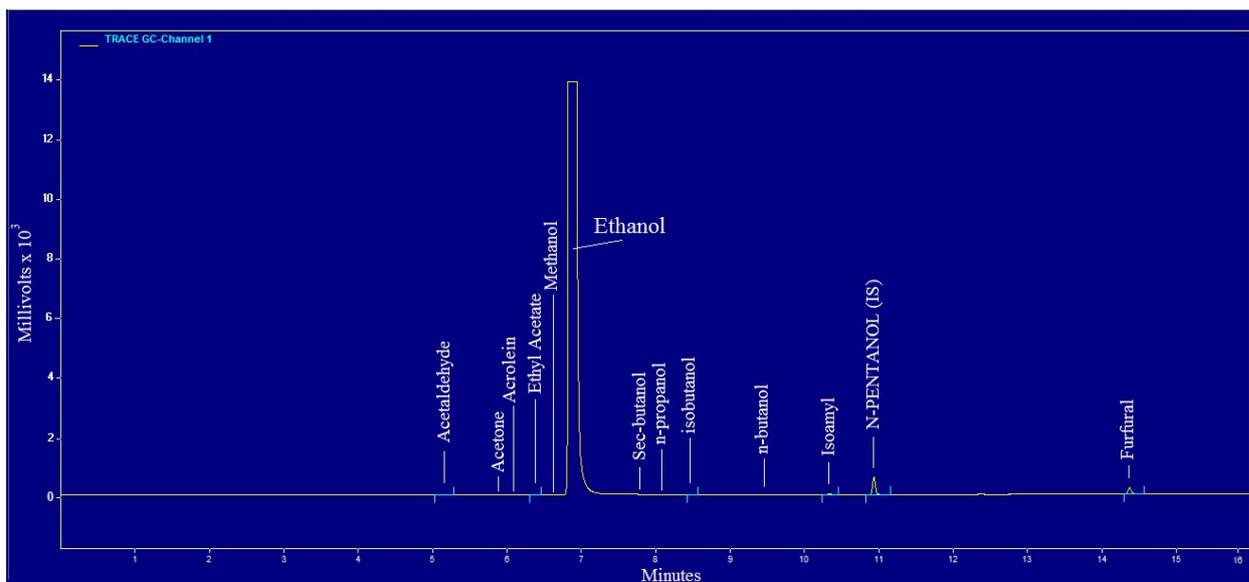


Figure ESI3

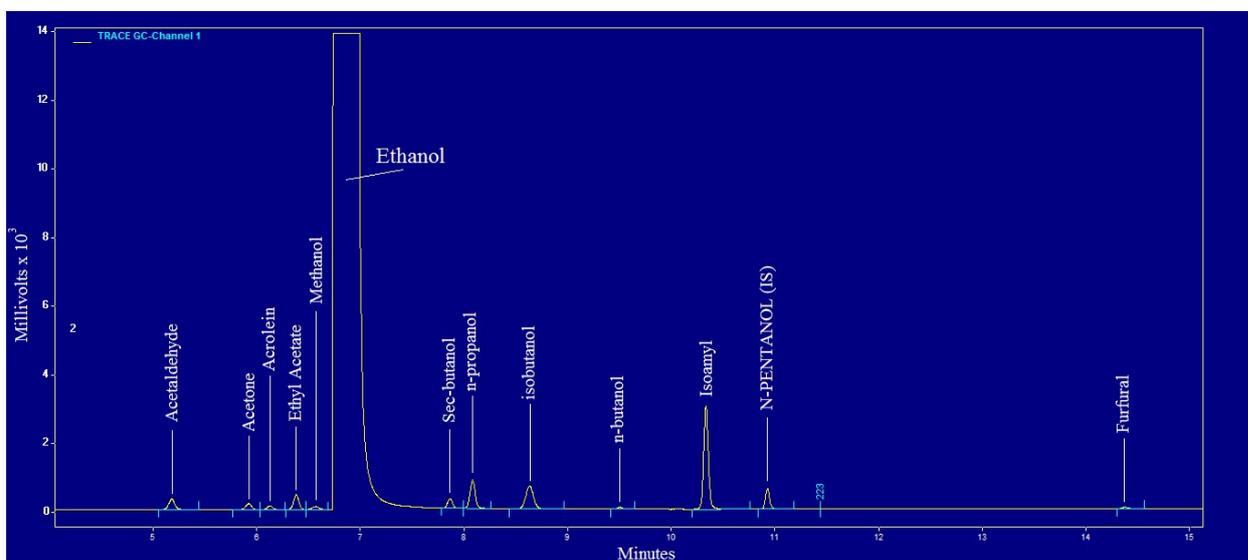


Figure ESI4

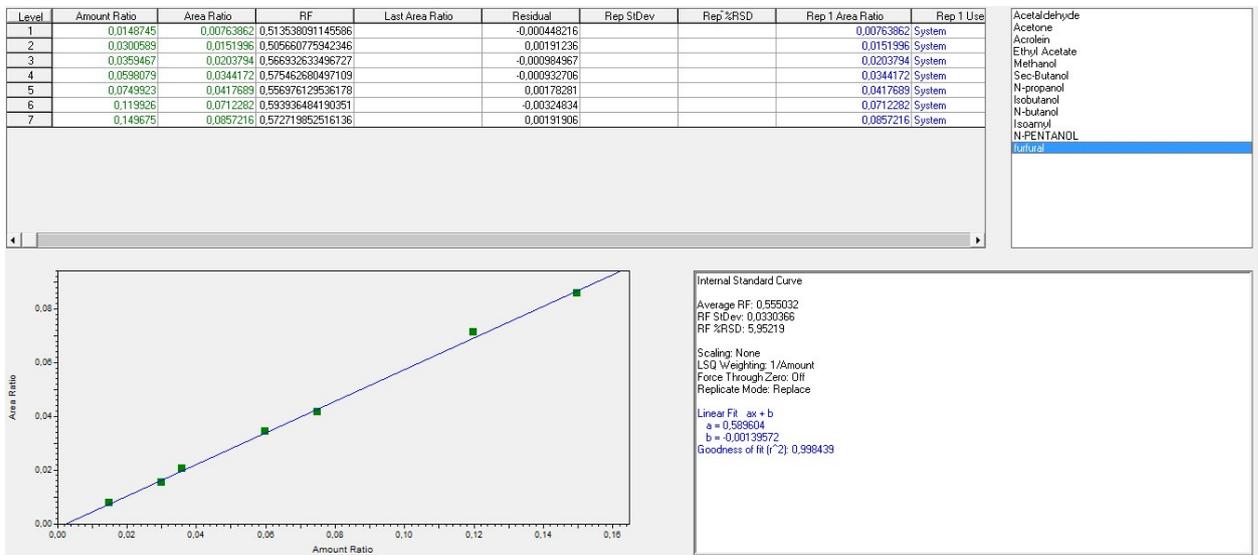


Figure ESI5

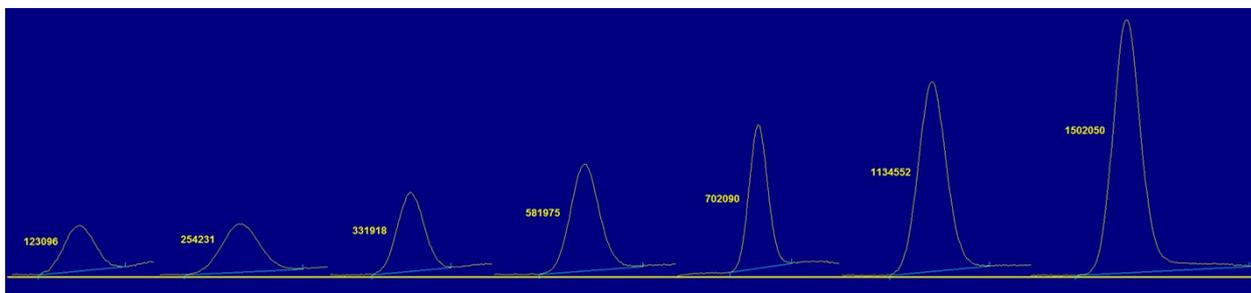


Figure ESI6

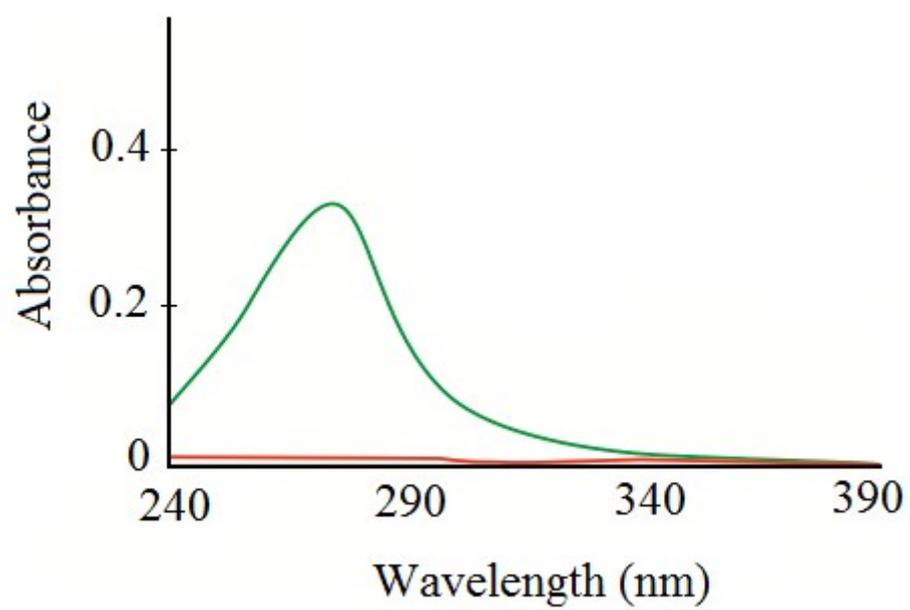


Figure ESI7

